Anti-Oxidative Effects of Vitamin B₂-Butyrate on the Cardiac Mitochondrial Disorders Induced by Adriamycin

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Summary The combined use of CoQ₁₀ with adriamycin has been recommended for reduction of the cardiotoxicity that occurs during cancer chemotherapy. Vitamin B₂-butyrate was also investigated in order to determine anti-oxidative effects on adriamycin cardiotoxicity. This vitamin analysis prevented enhanced lipid peroxidation and rectified the respiratory disorders of heart mitochondria induced by adriamycin, however, the deficiency of the CoQ₁₀-pool was not rectified. The combined approach of using CoQ₁₀ for rectifying the deficiency of this component and of using B₂-butyrate for reducing lipid peroxidation was indicated for adriamycin cancer chemotherapy.

Key Words vitamin B₂, adriamycin, mitochondria, lipid peroxide, CoQ₁₀

Adriamycin (ADM) is presently one of the most widely used agents for the treatment of solid tumors. Unfortunately, its clinical use has been compromised by an usual and potentially lethal cardiac toxicity. The cardiotoxicity of ADM is known, at least in part, to be mediated through adverse effects on mitochondrial function (3). In our previous paper (6), administration of ADM induced the uncoupling of oxidative phosphorylation and lipid peroxide formation in cardiac mitochondria. High-pressure liquid chromatographic analysis revealed that coenzyme Q₁₀ (CoQ₁₀) content decreased markedly in cardiac mitochondria of rats treated with ADM. The administration of exogenous CoQ₁₀ was able to restore CoQ₁₀-levels to normal, relieving respiratory disorders and preventing lipid peroxide formation in cardiac mitochondria. On the contrary, an excess of a single administration of CoQ₁₀ tends to enhance lipid peroxidation, owing to the quinone structure within the CoQ molecule (2). In order to reduce the lipid peroxide formed

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by ADM-treatment, it appeared advantageous to add a structurally different antioxidant with CoQ₁₀. Riboflavin-2',3',4',5'-tetrabutryate (B₂-butyrate) was selected for this study.

MATERIALS AND METHODS

Adriamycin was purchased from Kyowa Hakko Kogyo Company, Ltd. (Tokyo) and was dissolved in distilled water before use. Riboflavin-2',3',4',5'-tetrabutryate (B₂-butyrate) was supplied by Tokyo Tanabe Company Ltd. (Tokyo). B₂-Butyrate was dissolved in 10% ethanol, and diluted with olive oil until a final concentration of 0.8% B₂-butyrate was achieved. 2,3,6-Trimethyl-5-nonaprenyl-1-4, benzoquinone (TQ-9) was used for the purpose of quantitative determination of coenzyme Q (CoQ) homologues as an internal standard in high-pressure liquid chromatographic analysis (10).

Four groups of ten male rats were used, the first group daily receiving intraperitoneally ADM (4 mg/kg body weight) alone for one week. The second group received an intraperitoneal injection of B₂-butyrate (20 mg/kg body weight) daily. The third group received both ADM and B₂-butyrate. The last batch of animals served as controls.

The animals were killed by decapitation on the 7th day following administration of ADM and/or B₂-butyrate. Mitochondria were isolated from heart tissue by differential centrifugation after Nagarse digestion (6, 7). A Clark oxygen electrode was used to measure oxygen utilization polarographically using succinate as substrate (8). Respiratory responses are represented as State 3 and State 4 respiration, respiratory control ratio (RCR) and ADP/O ratio. Lipid peroxide content was determined using the thiobarbituric acid-acetic acid reaction, and expressed as nanomoles malondialdehyde per mg protein (5). CoQ homologues were extracted from cardiac mitochondria using *n*-hexane after saponification, and analyzed by a method of high-pressure liquid chromatography on a LiChrosorb RP-18 column as described in our previous paper (12). The protein content of the mitochondrial suspension was determined using the biuret reaction (1).

RESULTS

The lipid peroxide content of mitochondria from non-treated control animals and from ADM and/or B₂-butyrate treated animals is shown in Fig. 1. The administration of ADM resulted in the increase of lipid peroxide content in cardiac mitochondria ($p < 0.01$). The lipid peroxide content in mitochondria from rats treated with ADM and B₂-butyrate was not as high as that in mitochondria from animals treated with ADM alone. The combined use of B₂-butyrate with ADM significantly reduced the lipid peroxide formation induced by ADM ($p < 0.01$).

The respiratory responses of cardiac mitochondria influenced by administration of ADM and/or B₂-butyrate are shown in Table 1. The administration of

Fig. 1. Lipid peroxide content of cardiac mitochondria from rats treated with adriamycin (4 mg/kg) and/or B₂-butyrate (20 mg/kg) intraperitoneally daily for one week. Lipid peroxide contents are expressed as nanomoles of malondialdehyde per mg of mitochondrial protein.

Table 1. Influence of vitamin B₂-butyrate on respiratory response of cardiac mitochondria of rats treated with adriamycin.^a

Animals	Oxygen consumption ^b		RCR ^c	ADP/O ^d
	State 3	State 4		
Non-treated control	210.6 ± 12.7	46.9 ± 2.2	4.50 ± 0.45	1.51 ± 0.09
Adriamycin	164.5 ± 8.4	48.9 ± 7.4	3.43 ± 0.57	1.42 ± 0.12
Adriamycin and B ₂ -butyrate	189.2 ± 12.4	44.5 ± 4.1	4.26 ± 0.14	1.49 ± 0.11
B ₂ -butyrate	228.9 ± 2.6	53.0 ± 2.0	4.31 ± 0.13	1.39 ± 0.09

^a Respiratory responses of cardiac mitochondria from non-treated control and from rats treated intraperitoneally with adriamycin (4 mg/kg body wt.) and/or B₂-butyrate (20 mg/kg body wt.) daily for one week. Ten male rats were used in each group. ^b The values given for oxygen consumption are expressed as nanoatoms of oxygen utilized per min per mg mitochondrial protein, using succinate as substrate. ^c RCR (respiratory control ratio) is the quotient of the State 3 respiration value and the succeeding State 4 respiration value. ^d ADP/O ratio is the molar ratio of the ADP added to the consumed oxygen in State 3.

ADM resulted in a significant decrease in State 3 respiration ($p < 0.01$). It is clear from statistical calculation that the combined use of B₂-butyrate with ADM elevated State 3 oxygen consumption more than that with ADM administration alone ($p < 0.01$). ADM administration gave a lower value in RCR than with non-treatment ($p < 0.05$). Using B₂-butyrate, the RCR value increased significantly ($p < 0.05$) toward a normal level. In the animals co-treated with ADM and B₂-butyrate, the respiratory responses were close to normal. The combined use of B₂-butyrate with ADM appears to reduce the uncoupling of oxidative phosphorylation

Table 2. Content of CoQ homologues in cardiac mitochondria of rats treated with adriamycin and vitamin B₂-butyrate.^a

Animals	CoQ homologue ^b		
	CoQ ₈	CoQ ₉	CoQ ₁₀
Non-treated control	0.40 ± 0.05	9.56 ± 0.45	0.54 ± 0.07
Adriamycin	0.43 ± 0.12	8.56 ± 1.32	0.41 ± 0.06
Adriamycin and vitamin B ₂ -butyrate	0.45 ± 0.03	9.31 ± 0.75	0.47 ± 0.04
Vitamin B ₂ -butyrate	0.41 ± 0.08	10.10 ± 0.95	0.59 ± 0.05

^aCoQ₈, CoQ₉, and CoQ₁₀ content in cardiac mitochondria of rats treated with adriamycin (4 mg/kg) and/or B₂-butyrate (20 mg/kg) intraperitoneally daily for one week. ^bCoQ homologues from cardiac mitochondria were analyzed using a method of high-pressure liquid chromatography on a column of LiChrosorb RP-18 (4.5 × 150 mm) using ethanol : water (97:3 v/v) as mobile phase at a flow rate of 0.5 ml/min. The effluent was monitored at 257 nm. 2,3,6-Trimethyl-5-nonaprenyl-1,4-benzoquinone was used as internal standard. Values for CoQ homologues are expressed as µg per mg protein and represent the average ± SD of 5 determinations.

induced by ADM administration alone.

The CoQ homologue content of cardiac mitochondria influenced by administration of ADM and/or B₂-butyrate is shown in Table 2. It is apparent that administration of ADM resulted in a significant decrease in CoQ₁₀ content ($p < 0.01$). No significant difference was observed in CoQ₁₀ content between ADM-treated animals and B₂-butyrate co-treated animals ($p > 0.05$). Administration of B₂-butyrate was not effective in restoring the deficiency of CoQ₁₀ content in the cardiac mitochondria of rats treated with ADM.

DISCUSSION

We have shown that administration of ADM causes a significant deficiency of the CoQ₁₀-pool in rat cardiac mitochondria and involves a mitochondrial dysfunction (6). The therapeutic usefulness of CoQ₁₀ for preventing or minimizing the side-effect of cardiotoxicity induced by ADM was confirmed (6). In the recent finding of Myers *et al.* (4), the deleterious actions of ADM raised the possibility of at least two mechanisms of tissue damage: one which involves lipid peroxidation resulting in cardiac toxicity; another which involves the binding of ADM to the DNA molecule.

In our previous paper (6), the co-administration of exogenous CoQ₁₀ with ADM was confirmed to minimize lipid peroxidation in mitochondria and to relieve the respiratory disorders induced by ADM. Takeshige *et al.* (13) also demonstrated that reduced CoQ₁₀ functions as a potent antioxidant against membrane lipid peroxidation in submitochondrial particles. On the contrary, Boveris *et al.* (14)

documented that a reduced form of CoQ₁₀ produces small amount of superoxide anion which serves as an initiating factor of lipid peroxidation. Although Takeshige *et al.* (13) ruled out the direct possibility of generating superoxide anion by antioxidation of CoQ₁₀, the site of production is considered as being a region between mercury- and rotenone-sensitive sites in the respiratory chain. In our previous paper (2), it was demonstrated that administration of large amounts of CoQ₁₀ alone, more than 10 mg per kg body weight (not available for clinical use) for one week, involved enhanced lipid peroxidation in mitochondria. This discrepancy has not yet been resolved, but may be dependent on a quinone functional group of the CoQ₁₀ molecule. Therefore, the anti-oxidant compound not having quinone structure was studied in the present paper, to determine the anti-oxidative effect under ADM administration. The results reported in this paper indicate that the dose of CoQ₁₀ administration is only sufficient for reversing the deficiency of CoQ₁₀ induced by ADM.

Adriamycin contains both quinone and hydroquinone functional groups, suggesting possible activity in oxidation-reduction reactions. It is thought that such a process might be involved in the interaction of ADM with CoQ₁₀. This interaction would make complicated the understanding of the mechanism of the antioxidative effect of CoQ₁₀ against ADM.

It is well known that riboflavin (vitamin B₂) has an anti-oxidant role (15). B₂-Butyrate is a long-acting vitamin because of its hydrophobic structure (11). The anti-oxidative effect of B₂-butyrate for lipid peroxidation in ADM administration was found to be more effective than that of the B₂-free form or CoQ₁₀ (9). In our present paper, B₂-butyrate was able to restore lipid peroxide formation induced by ADM administration, and consequently the respiratory dysfunction was improved. However, the CoQ₁₀-pool in cardiac mitochondria was deficient, unlike the case of CoQ₁₀ administration. CoQ is one of the biologically active anti-oxidative compounds, and the anti-oxidative effect of CoQ₁₀ has been confirmed in our ADM experiment (6). However, for preventing lipid peroxidation induced by excess of CoQ itself, the combined use of B₂-butyrate with CoQ₁₀ has been recommended for minimizing the enhanced lipid peroxidation induced by ADM. CoQ is essential for reversing the deficiency of the CoQ-pool, and B₂-butyrate is effective for reducing lipid peroxide formation. Additional work is in progress to determine the dose rate for CoQ₁₀ and B₂-butyrate in administration (2). The combined use of anti-oxidant with CoQ₁₀ might be of therapeutic benefit for preventing side-effects in ADM cancer chemotherapy.

REFERENCES

- 1) Gornall, A. G., Bardawill, C. J., and David, M. M. (1949): Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.*, **177**, 751-766.
- 2) Katsuki, T., and Ogura, R. (1981): Effect of simultaneous administration of vitamin B₂-butyrate and ubiquinone-10 on heart mitochondrial disorder induced by adriamycin. *Vitamins* (in Japanese), **55**, 517-522.

- 3) Kishi, T., Kishi, H., and Folkers, K. (1977): Inhibition of cardiac CoQ₁₀-enzyme by clinically used drugs and possible prevention, in *Biochemical and Clinical Aspects of Coenzyme Q*, ed. by Folkers, K., and Yamamura, Y., Elsevier, Amsterdam, pp. 47–62.
- 4) Myers, C. E., McGuire, W. P., Liss, R. H., and Young, R. C. (1977): Adriamycin: The role of lipid peroxidation in cardiac toxicity and tumor response. *Science*, **197**, 165–167.
- 5) Nagata, O., Sayanagi, H., and Ogura, R. (1978): Improved method for the determination of lipid peroxide in the isolated mitochondria of rat liver. *J. Kurume Med. Assoc.* (in Japanese), **41**, 292–298.
- 6) Ogura, R., Toyama, H., Shimada, T., and Murakami, M. (1979): The role of ubiquinone (Coenzyme Q₁₀) in preventing adriamycin-induced mitochondrial disorders in rat heart. *J. Appl. Biochem.*, **1**, 325–335.
- 7) Ogura, R., Toyama, H., Nagata, O., Ono, T., Shimada, T., and Murakami, M. (1979): Biochemical and scanning electron microscopic properties of mitochondria isolated from rat cardiac muscle. *Kurume Med. J.*, **26**, 51–56.
- 8) Ogura, R., Sayanagi, H., Noda, K., Hara, H., Shimada, T., and Murakami, M. (1979): Biochemical and morphological changes in isolated rat liver mitochondria exposed to ultraviolet light. *Kurume Med. J.*, **25**, 65–75.
- 9) Ogura, R., Toyama, H., and Katsuki, T. (1979): Effect of vitamin to the mitochondrial disorders induced by lipid peroxide formation (adriamycin). *Vitamins* (in Japanese), **53**, 569–576.
- 10) Ruegg, R., Gloor, U., Goel, R. N., Ryser, G., Wiss, O., and Isler, O. (1959): Synthesis of ubiquinone (45) and ubiquinone (50). *Helv. Chim. Acta*, **42**, 2616–2621.
- 11) Tahara, K., Matsuoka, S., and Ohama, H. (1974): Effect of riboflavin and riboflavin-2',3',4',5'-tetrabutryrate on rat liver microsomal lipid peroxidation. *J. Nutr. Sci. Vitaminol.*, **20**, 81–88.
- 12) Toyama, H., Hara, H., Ono, T., and Ogura, R. (1979): Determination of CoQ homologues in rat heart mitochondria by high-pressure liquid chromatography. *Kurume Med. J.*, **42**, 164–173.
- 13) Takeshige, K., Takayanagi, R., and Minakawa, S. (1980): Reduced coenzyme Q₁₀ as an antioxidant of lipid peroxidation in bovine heart mitochondria, in *Biomedical and Clinical Aspects of Coenzyme Q₁₀*, Vol. 2, ed. by Yamamura, Y., Folskers, K., and Ito, Y., Elsevier/North-Holland Biomedical Press, pp. 15–26.
- 14) Cadenas, E., Boveris, A., Ragan, I., and Stoppani, A. O. M. (1977): Production of superoxide radicals and hydrogen peroxide by NADH-ubiquinone reductase and ubiquinone-cytochrome *c* reductase from beef-heart mitochondria. *Arch. Biochem. Biophys.*, **180**, 248–257.
- 15) Saito, Y., Ohishi, N., and Yagi, K. (1981): Protective effect of riboflavin on suppression of growth caused by oxidized oil. *J. Nutr. Sci. Vitaminol.*, **27**, 17–21.